

#### IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Joseph M. Jilka

ART UNIT: 1635

SERIAL NO: 10/086,062

EXAMINER: Epps, J.

FILED:

February 28, 2002

TITLE:

NOVEL PLANT PROMOTER SEQUENCES AND METHODS OF USE

FOR SAME

#### 131 DECLARATION OF JOSEPH M. JILKA

Commissioner of Patents and Trademarks Washington, D.C. 20231

#### Dear Sir:

I, Joseph M. Jilka hereby declare the following:

- 1. That I am the inventor for the above-identified patent application; that I conceived and reduced to practice in the United States the invention claimed in the above-identified patent application prior to the international publication date of March 23, 2000, of the cited PCT Application No. WO 00/15810 to Goldsbrough as evidenced by the enclosed notebook pages.
- 2. Attached Exhibit A is a copy of notebook records relating to this conception wherein construction of proposed versions of the ubiquitin variants show a no heat shock version. Also relating to this conception is Exhibit B which is a copy of a table listing the promoters made which show a no heat shock version. Attached Exhibit C are primers among which is the no heat shock version, version 4A, 4B.
- 3. That pursuant to this conception, I actually reduced to practice in the United States the invention claimed in the above-identified patent application prior to March 23, 2000, the international publication date of the cited Goldsbrough patent. Attached Exhibit D and E are copies of the notebook records of Kathy Beifuss, who worked under my direction and supervision, however, did not contribute materially to the above-identified invention, relating to the actual reduction to practice, wherein Exhibit D shows use the no heat shock

version in a mini-prep and Exhibit E shows use of the no heat shock version in sequencing. Additionally, attached Exhibits F and G relating to the actual reduction to practice is a copy of the notebook records of Chris Brooks and Elizabeth Wilfong, both who worked under my direction and supervision, however, did not contribute materially to the above-identified invention, showing the GUS reporter gene expression in corn seed using the Ubi promoter variant, GSC, the ubiquitin promoter having no heat shock elements. Wherein total soluble protein (1µg) was incubated in 100µl lysis buffer and the reaction initiated with 5mM 4methylumbelliferyl β-D-glucuronide (MUG). The reaction was incubated for up to about 20 minutes at 37°C. At specific time points approximately 25µl of volume of the reaction mixture was transferred into a reading plate that had 175µl of Stop buffer in the well. The reaction plate was placed at 37°C until the next time point. Generally readings at 0, 15, 30, and 60 minutes were taken. Plates were read at 360nm excitation wavelength and 460 nm emission wavelength. GUS protein levels were then calculated by comparison to a standard curve of 1-100µM 4-methylumbelliferyl. Exhibit G shows results from a 10 minute reading. The dates of these records have been redacted, however, the acts of conception and reduction to practice occurred prior to March 23, 2000, the international publication date of the cited Goldsbrough patent.

- 4. That Exhibits, A, B, C, D, E, F, and G, which relate to the aforementioned conception and reduction to practice, correspond to the invention disclosed and claimed in the above-identified patent application.
- 5. The undersigned further declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issuing thereon.

Date: 7/17/02

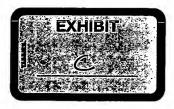
Joseph M. Jilka

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-1					
S S S S	maize polyubiquitin 1 (UBII)	90	DPHP8904	C00	ASS.
		GUS-boths	OPGN7062	Con	85
SNOR	malze gobuln 1	SIS ouris	OPGN9075	E 0	
SCND13	PGNpr3 Imaize 22 kD aipha-zuén	CLS darts	OPGN0071	200	58
<b>KSNO</b> R4	make UBI) no heat shock elements (HCE): UbiC	GIS Septs	oPGN7547	Co.	300
SND15	PGNpc5 Implue UBIT no 3º HSE: Upilis	O.C. Cartes	D/GN/565	5	8
GNOr6	PIGNOR'S IMOZZE UBIT NO S'HISE UDIE	QUS-baths	DPGN7583	COL	5
ZONO1	mabe UBIT no HOS averlaps; Utalf	GUS-boths	PPGN7400	S	380
Syg B	thatre UBIT replace HSE with 3x Pt.1 seed specific coment; Ubits	GUS-bark	PPGN8926	(S)	989
\$2 \$2	leasinte polyutiquilin 1	GUS-60018	DPCN8084	Š	esi
SNoria	teosinte polyubiquitin la	GUS-64th	DPGN8985	S	30
SNP11	PGNpill sorghum polyubladin i	GUS-AXTE	DPGN8986	S	380
SNP/12	PGNpr12[maize glutathiane-S-transferase I (GSTI)	GUS-Arth	DPGNR987	5	80
SNOT	synthetic promoter RsynD with 355 enhancer 6' (tested with mote Adh-1 into	GUS GADIE	pPGN9005	E C	25
Shorts	PGNot14 syntholic promoter Asyn7 with 365 enhancer 5' (tested with mate Ach-1 Intri	CUS Outris	pPGN9007	Son	33
GNOVIS	PGNort Smote HNGP	GUS-60016	DPGN9016	5	ğ
Shale	PGNat16 make P promoter (tested with make Adh-1 intron)	GUS-60this	pPGN9035	80	8
ZVD.	PGN/xx17 [modified version of Agro mannophie synthose (suporMAS)	GUS	pPHP10336	Peo	3
8 045	Kayar i Bibean physeain	GUS-10xas	GUS-10kasp/pPCN0275	Peo	8
	·	GUS-BATIS	pPGN5690	Ped	ž
	QI:	GUS-BUNS	PPGN9042	CO CO	150
SYDLY?		GUS-bartis	pPGN9056	<u>ව</u>	¥¥
2005		GLS CAPIS	pPGN9057	S S	88
22/2/2		GLS-6whis	DPGN9060	8	Ş S
13000	PSASO(2) Imalze globulin 2	GLS-AMB	PPGN9076	Š	



# GIBCO BRL Custom Primers Certificate of Analysis

Primer Name: UBI HSP VER. 1A		Primer Number: Al	3333C10 (C10)
Illinot reality,		Primer Length:	66
Researcher:	CGG CAT CTC TGT CGC	TGC CTC CAC CGT TGG ACT TG	C TCC GCT
GTC GGC ATC	CAG AAA T		
	21299.2	· μg per OD:	<b>3</b> 1.3
Molecular Weight µg/µmole:	678.6	nmotes per OD:	1.4
Millimolar Extinction Coefficient:			39.3
Purity	Desailed	00'6	1234
Tm (1 M Na+)	96	hã,ə.	
Tm (50 mM Na+)	76	nmotes	
% GC	60	Coupling Eff.	99%
Notes:			
Primer Name: UBI HSP VER.18 Researcher: Sequence (5' to 3'): PTT TCT GGA	TGC CGA CAG CGG AGC	Primer Length: AAG TCC AAC GGT GGA GGC A	67 GC GAC AGA
GAT GCC GTC	G CCG TCT GC		
Molecular Weight µg/µmole:	21897.4	. µg per OD:	29.8
Millimolar Extinction Coefficient	<b>732.9</b> .	nmoles per OD:	1.3
Purity	Deserred	OD's	10.7
- VV	97	ħ8, <b>e</b> ,	319
Tm (1 M Na+)		nmoles	14
Tm (1 M Na+) Tm (50 mM Na+)	76	411110-10-1	**
Tm (1 M Na+) Tm (50 mM Na+) % GC	76 62	Coupling Eff.	. 89%

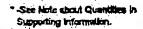


"-See Note about Quantities in Supporting Information.



# GIBCO BRL Custom Primers Certificate of Analysis

		•	e se
Primer 1:		<b>.</b>	1000 (200
Primer Name: UBI HSPA VER.2A		Primer Number: D037	3807 (B07)
Researcher		Primer Length:	81
	ncg gca tet etg teg : etc ege tgt egg cat	ctg cct ctg gac ccc tct cga cca gaa at	CCA CCG
Môlecular Weight µg/µmole:	26105.2	µg per QD:	31.6
Millimolar Examption Coefficient:	824.3	nmoles per OD:	1.2
Purity .	Desatt	QD's	90.0
Tm (1 M Na+)	98	hā,e.	2850
Tm (50 mM Na+)	77	nmoles	108
% GC	61	Coupling Eff.	98%
Notes:		10\$0_0	المدارات و ووا
Primer 2:		·	
Primer Name: UBI HSPB VER.28		Primer Number, D03	73B06 (808)
Researcher:		Primer Length:	82
Molecular Weight µg/µmole:	CGA CAG AGA TGC CG 26872.4	µg per OD:	29.7
Millimoter Extraction Coefficient:	902.2	nmoles per OD:	1.1
Purity	Desalt	OD's	77.0
Tm (1 M Ne+)	99	hã, e.	2294
Tm (50 mM Na+)	78	nmoles	<b>6</b> 5
% GC	63	Coupling Eff.	98%
Notes:		730	المراسم معالم
Primer 3:			
Primer Name: UBI HSPA VER.34	<b>\</b>	Primer Number: D03	73B09 (B09)
Researcher:		Primer Length:	B1
		CTG CCT CTC GAG AGT TCC GCT	CCA CCG
	CTC CGC TGT CGG CAT		
Molecular Weight µg/µmote:	26160.2	µg per OD:	31.5
Millimolar Extinction Coefficient:	830.6	nmoles per OD:	1.2
Purity	Desalt	OD's	88.7
Tm (1 M Ne+)	98	ha.e.	2783
Tm (50 mM Na+)	76	nmoles	106



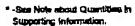




Coupling Eff.

## **GIBCO BRL Custom Primers** Certificate of Analysis

<u>rimer 4:</u> Primer Name;     UBI HSPB VER.3I	В	Primer Number: D0373B1	0 (B10)
rimer rame, obi hor by circo. Researcher:	•		B2
researcher.	ATG CCG ACA GCG GAG	CAA GTC CAA CGG TGG <u>AGC GGA AC</u>	
GAG AGG CAG	G CGA CAG AGA TGC CG	GCC GTC TGC	
Actecular Weight ug/pmole:	26816.4		9.7
Allimolar Extinction Coefficient:	901.3		1.1
Purity	Desalt	OD's 8	3.2
īm (1 M Na+)	99	μο'ε' 24	76
rm (50 mM Na∸)	77	nmoles	92
% ec	62	Coupling Eff. 9	<b>8%</b>
Notes:		هد غير عده	المراسع ١٥٥
			<u> </u>
rimer S:			
Primer Name: UBI HSPA VERA	A	Primer Number: D03738	11 (B11)
Researcher.			96
Sequence (5' to 3");P-A GAC GGC	ACO GCA TCT CTG TCG	CTG CCT CTG GAC CCC TCT CGA CTC	GAG
AGT TCC GCT	r cca ccg ttg gac ttg	CTC CGC TGT CGG CAT CCA GAA AT	
AGT TCC GCT Molecular Weight µg/µmole:	r cca ccg ttg gac ttg 30986.2	CTC CGC TGT CGG CAT CCA GAA AT  pg per OD;  3	1.7
AGT TCC GCT Woleoular Weight µg/µmole:	r cca ccg ttg gac ttg	CTC CGC TGT CGG CAT CCA GAA AT  µg per OD:  nmoles per OD:	1.7 1.0
AGT TCC GCT Molecular Weight µg/µmole: Millimolar Extinction Coefficient:	r cca ccg ttg gac ttg 30986.2	CTC CGC TGT CGG CAT CCA GAA AT  pg per OD:  nmoles per OD:  OO's	1.7 1.0
AGT TCC GCT Molecular Weight µg/µmole: Millimolar Extinction Coefficient: Purity	r CCA CCG TTG GAC TTG 30986.2 976.3	CTC CGC TGT CGG CAT CCA GAA AT  pg per OD:  nmoles per OD:  OO's	1.7 1.0 19.3
AGT TCC GCT Molecular Weight µg/µmole: Millimolar Extinction Coefficient: Purity Tm (1 M Na+)	r CCA CCG TTG GAC TTG 30988.2 978.3 Desait	CTC CGC TGT CGG CAT CCA GAA AT  µg per OD:  nmoles per OD:  OO's  µg's'  nmoles	1.7 1.0
AGT TCC GCT Molecular Weight µg/µmole: Millimolar Extinction Coefficient: Purity Tm (1 M Na+) Tm (50 mM Na+)	r CCA CCG TTG GAC TTG 30986.2 976.3 Desalt 100	PETC CGC TGT CGG CAT CCA GAA AT  pg per OD:  nmoles per OD:  OO's  pg's' 2  nmoles	1.7 1.0 19.3
AGT TCC GCT Molecular Weight µg/µmole: Millimolar Extinction Coefficient: Purity Tm (1 M Na+) Tm (50 mM Na+) % GC	7 CCA CCG TTG GAC TTG 30986.2 976.3 Desalt 100 78	CTC CGC TGT CGG CAT CCA GAA AT  µg per OD:  nmoles per OD:  OO's  µg's'  nmoles	1.7 1.0 9.3 833 91
AGT TCC GCT Molecular Weight µg/µmole: Millimolar Extinction Coefficient: Purity Tm (1 M Na+) Tm (60 mM Na+) % GC Notes;	7 CCA CCG TTG GAC TTG 30986.2 976.3 Desalt 100 78	PETC CGC TGT CGG CAT CCA GAA AT  pg per OD:  nmoles per OD:  OO's  pg's'  nmoles  Coupling Eff.	1.7 1.0 9.3 833 91
AGT TCC GCT Molecular Weight µg/µmole: Millimotar Extinction Coefficient: Purity Tm (1 M Na+) Tm (50 mM Na+) % GC Notes: Primer 6:	r CCA CCG TTG GAC TTG 30986,2 978.3 Desalt 100 78 61	Proper Code CAT CCA GAA AT pg per OD:  nmoles per OD:  OO's  pg's'  nmoles  Coupling Ent.  9to pag	1.7 1.0 19.3 813 91
AGT TCC GCT Molecular Weight µg/µmole: Millimolar Extinction Coefficient: Purity Tm (1 M Na+) Tm (60 mM Na+) % GC Notes: Primer 6: Primer Name: UBI HSPB VER.4	r CCA CCG TTG GAC TTG 30986,2 978.3 Desalt 100 78 61	Primer Number: 003738	1.7 1.0 9.3 813 91 18%
AGT TCC GCT Molecular Weight µg/µmole: Millimolar Extinction Coefficient: Purity Tm (1 M Na+) Tm (60 mM Na+) % GC Notes: Primer 6: Primer Name: UBI HSPB VER.4 Researcher:	7 CCA CCG TTG GAC TTG 30986.2 975.3 Desalt 100 78 61	Primer Number: 003738	1.7 1.0 19.3 813 91 18% 12 (B12)
AGT TCC GCT Molecular Weight µg/µmole: Millimolar Extinction Coefficient: Purity Tm (1 M Na+) Tm (60 mM Na+) % GC Notes: Primer 6: Primer Name: UBI HSPB VER.4 Researcher: Sequence (5' to 3):P-T TTC TGG	CCA CCG TTG GAC TTG 30986.2 978.3  Desalt 100 78 61  ATG CCG ACA GCG GAG	Primer Number: D03738 Primer Length:  CTC CGC TGT CGG CAT CCA GAA AT  pg per OD:  3 nmoles per OD:  2 nmoles  Coupling En.  9 piner Number: D03738  Primer Length:  CAA GTC CAA CGG TGG AGC GGA AG	1.7 1.0 9.3 813 91 18% 12 (B12) 97
AGT TCC GCT Molecular Weight µg/µmole: Millimolar Extinction Coefficient: Purity Tm (1 M Na+) Tm (50 mM Na+) % GC Notes: Primer 6: Primer Name: UBI HSPB VER.4 Researcher: Sequence (5' to 3):P-T TTC TGG GAG TCG AG	CCA CCG TTG GAC TTG 30986.2 978.3 Desalt 100 78 61 48 ATG CCG ACA GCG GAG	Primer Number: D03738 Primer Length: CAA GTC CAA GAA AT  PG CGA CAG AGA TGC CGT GCC GTC  CTC CGC TGT CGG CAT CCA GAA AT  PG PART  PART  PART  PG CGA CAG AGA TGC CGT GCC GTC	1.7 1.0 19.3 813 91 18% 12 (B12) 97 21 CTC
AGT TCC GCT Molecular Weight µg/µmole: Millimolar Extinction Coefficient: Purity Tm (1 M Na+) Tm (50 mM Na+) % GC Notes: Primer 6: Primer Name: UBI HSPB VER.4 Researcher: Sequence (5' to 3):P-T TTC TGG GAG TCG AG Molecular Weight µg/µmole:	CCA CCG TTG GAC TTG 30986,2 978,3 Desalt 100 78 61 48 ATG CCG ACA GCG GAG 31781,4	Primer Number: D03738 Primer Length: CAA GTC CAA GAG AGG GGA AGG GGA AGG GGA CAG AGA TGC CGT GCC GTC  Pg per OD:  OO's  Representation of the period of the	1.7 1.0 19.3 833 91 18% 12 (B12) 97 21 CTC 19.6
AGT TCC GCT Molecular Weight µg/µmole: Millimolar Extinction Coefficient: Purity Tm (1 M Na+) Tm (50 mM Na+) % GC Notes: Primer 6: Primer Name: UBI HSPB VER.4 Researcher: Sequence (5' to 3):P-T TTC TGG GAG TCG AG Molecular Weight µg/µmole: Millimolar Extinction Coefficient:	CCA CCG TTG GAC TTG 30986.2 978.3  Desalt 100 78 61  ATG CCG ACA GCG GAG A GGG GTC CAG/AGG CA 31791.4 1070.6	Primer Number: D03738 Primer Length: CAA GTC CAA GAG AGG GGA AGG AGG GGA AGG AGG GGA AGG A	1.7 1.0 9.3 813 91 18% 12 (B12) 97 21 CTC 1GC 19.6 0.9
AGT TCC GCT Molecular Weight µg/µmole: Millimolar Extinction Coefficient: Purity Tm (1 M Na+) Tm (50 mM Na+) % GC Notes: Primer 6: Primer Name: UBI HSPB VER.4 Researcher: Sequence (5' to 3):P-T TTC TGG GAG TCG AG Molecular Weight µg/µmole: Millimolar Extinction Coefficient: Purity	CCA CCG TTG GAC TTG 30986,2 978,3  Desalt 100 78 61  ATG CCG ACA GCG GAG A GGG GTC CAG/AGG CA 31791,4 1070,6  Desalt	Primer Number: D03738 Primer Length: CAA GTC CAA GAA AT  Per CAA GTC CAA CAG AGA AGA AGA AGA TGC CGT GCC GTC  Per CAA GTC CAA CAG AGA TGC CGT GCC GTC  Per CAA GTC CAA CAG AGA TGC CGT GCC GTC  Per CAA GTC CAA CAG AGA TGC CGT GCC GTC  Per CAA GTC CAA CAG AGA TGC CGT GCC GTC  Per CAA GTC CAA CAG AGA TGC CGT GCC GTC  Per CAA GTC CAA CAG AGA TGC CGT GCC GTC  Per CAA GTC CAA CAG AGA TGC CGT GCC GTC  Per CAA GTC CAA CAG AGA TGC CGT GCC GTC  Per CAA GTC CAA CAG AGA TGC CGT GCC GTC  Per CAA GTC CAA CAG AGA TGC CGT GCC GTC  Per CAA GTC CAA CAG AGA TGC CGT GCC GTC  Per CAA GTC CAA CAG AGA TGC CGT GCC GTC  Per CAA GTC CAA CAG AGA TGC CGT GCC GTC  Per CAA CAG TGC CGT GCC GTC TGC TGC TGC TGC TGC TG	1.7 1.0 9.3 833 91 98% 9 1 - 9 - 9 12 (B12) 97 12 (CTC) TGC 29.6 0.9
AGT TCC GCT Molecular Weight µg/µmole: Millimolar Extinction Coefficient: Purity Tm (1 M Na+) Tm (50 mM Na+) % GC Notes: Primer 6: Primer Name: UBI HSPB VER.4 Researcher: Sequence (5' to 3):P-T TTC TGG GAG TCG AG Molecular Weight µg/µmole: Millimolar Extinction Coefficient: Purity Tm (1 M Na+)	T CCA CCG TTG GAC TTG 30986.2 978.3  Desalt 100 78 61  ATG CCG ACA GCG GAG A GGG GTC CAG/AGG CA 31791.4 1070.6  Desalt 100	Primer Number: D03738 Primer Length: CAA GTC CAA CAG TGC GGA AS AG CGA CAG AGA TGC CGT GCC GTC  Page Per OD:  CAB GTC CAA CGG TGG AGC GGA AS AG CGA CAG AGA TGC CGT GCC GTC  Pag per OD:  CAB CGG TGG AGC GGA AS AG CGA CAG AGA TGC CGT GCC GTC  Pag per OD:  CD's  Page CGA CAG AGA TGC CGT GCC GTC  Page CGA CAG CGG TGG CGT GCC GTC  Page CGA CAG CGG TGG CGT GCC GTC  Page CGA CAG CGG TGC CGT GCC GTC  Page CGA CAG CGG TGG CGT GCC GTC  Page CGA CAG CGG TGC CGT GCC GTC  Page CGA CAG CGC CGT GCC GTC  Page CGA CGC CGC CGT GCC GTC  Page CGA CGC CGC CGC CGC CGC CGC CGC CGC CGC	1.7 1.0 19.3 833 91 18% 12 (B12) 97 21 CTC 15C 19.6 0.9
AGT TCC GCT Molecular Weight µg/µmole: Millimolar Extinction Coefficient: Purity Tm (1 M Na+) Tm (60 mM Na+) % GC Notes: Primer 6: Primer Name: UBI HSPB VER.4 Researcher: Sequence (5' to 3):P-T TTC TGG GAG TCG AG Molecular Weight µg/µmole: Millimolar Extinction Coefficient: Purity	CCA CCG TTG GAC TTG 30986,2 978,3  Desalt 100 78 61  ATG CCG ACA GCG GAG A GGG GTC CAG/AGG CA 31791,4 1070,6  Desalt	Primer Number: D03738 Primer Length: CAA GTC CAA CAG TGC GGA AS AG CGA CAG AGA TGC CGT GCC GTC   pg per OD:  nmoles  Primer D03738  Primer Length: CAA GTC CAA CGG TGG AGC GGA AS AG CGA CAG AGA TGC CGT GCC GTC   pg per OD:  nmoles per OD:  pg's"  pg's"  nmoles	1.7 1.0 9.3 833 91 98% 9 1 - 9 - 9 12 (B12) 97 12 (CTC) TGC 29.6 0.9



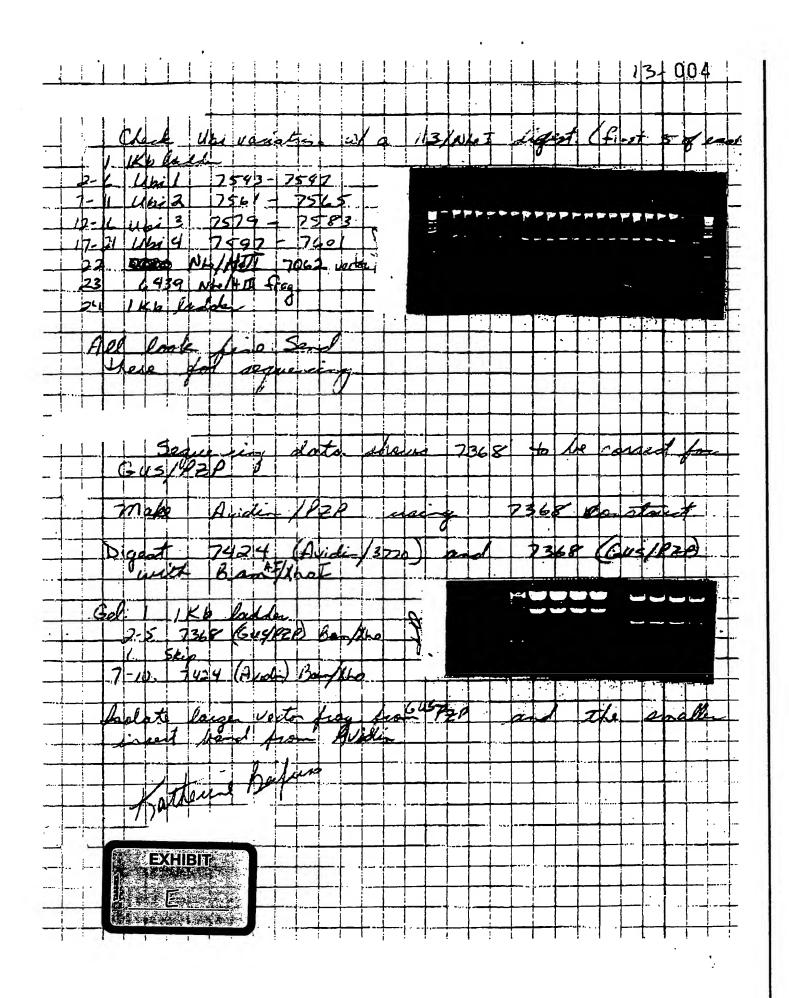
Notes:





Nucleoto o 5596 4216 5577 Digest 5596 a Smalle sure; 42181 4/2 B 16860 atas Diapor 5596 RAHSS: NA Mini-propo K Heal





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	<ul> <li>4 - 11 p. (a) the second sector (a) (b) specifically the discrete behavior of the second sector (a) in the sector (a) in the second sector (a) in the sector (a) in the second sector (a) in the sec</li></ul>		• • • • • •	e e e e e e e e e e e e e e e e e e e
Purpose: T	O QUANTITATE THE	AMOUNT OF GUS	IN Corn	Leed
MATERIALS:	REACTION PLATE-	· CosTAR ELA	RIA	
	READING PLATE -	Nunc Flaver	runc Pory:	Soel
	Mu- 4 Hemmu	un BELLI FERONE	E (SIGMA	H-1508)
	MUG- 4 METHILL			
	MICROBALANCE		0,111,000,000,	
	FLUORISCENCE MI	CECPHATE REA	DEC.	
				•
PROCEDURE:	USE PROTOCON F	Towns on Pace	6# 5M	OF THE
	NOTEBOOK (#58)		/	7/11/2
RESULTS:	DATA FOUND B	ELOW. (BASET	> 0~0 ZD	-Mæio
	READINGS.			, · · · · · ·
Supret	°20 TSP_	Shoulke	GOTSP	
	. <i>0</i> 88		0.087	
-5	••	-2	0.54	•
SSD 01120-1		-3	0.61	
-2	, W	-4	0.10	
3	170		0.06	
		11 0808 -1	0.001	\ \ \.
-5	ND	-Z	0.002	
GSE 15070-4	0.28	~3	0.007	Χ
11 05050-1	0.17	-4	ND	
2	0.015		0.001	/ \
-3	0.010	11 07050 -1	0.3	
-4	0.174	-2	0.089	
-5	0.010	-3	8,27	
11 05090-1	0.043	-4	0.013	
-2	0.014	-5	0.43	
-3	0.001	Ŭ	V.7~	٠.
-4	0.001			
5	0.004	Investigato	)r.	Book # 58
SSC 01010-1	0.006 EXHERT	mvostigati	л. ^ ^	DOUK # 20.
-2	0.0/0		Burn	Date:
-3	0.009		72 000V	<i>ω</i> αισ: .
-4	0.60	Witness:	Valle de	
-5	CEPT SECRETARY	- willed	n widout	_Date:

### Gus Assay

-3

-4

-5

4-2 0.4

4.5 0.8 4.5 0.8

# DEE PURPOSE, MATERIALS, & PROCEDURE BELOW.

CB Rossing Plate-Nunc Fluoronum: Polysorp 96-well black plate MU 4-methylumbelliforone (Signa M-1508) MUG 4-methylumbelliforone 8-glucuronide (Signa M-9130) Dilute the 20 mM MUG substrate stock to 5 mM with lysis buffer. Add 15 µl of 5 mM MUG to every well including both standard and sample wells and unit to start the reaction. Immodistely after adding the MUG. 5 at of notation from the reaction plate into a proposed reading the MUS 5 at of notation from the reaction plate into a proposed reading ance the reaction plate at 3 TC usual the next time point. At each set time point, parcie 25 at of a solution from the reaction plate pured reading plate, is stable for reversal-bours since it has born stopped. Note that the reaction is essential for fluorescence formation. ephate pH 7.0, 1 mM EDTA, 10 mM Note: 50 mM sodium phosphate is made by mixing 97 ml of Stock A (0.2M Naftly-O. (27.6 g/L.) with 153 ml of Stock B (0.2M Naftly-O. (27.6 g/L.) with 153 ml of Stock B (0.2M Naftly-O. (27.6 g/L.) and bringing to a final volume of 1.0 L with 41.0.

Also note that the 10 mld 5ME should be added to an alliquot of the lysis buffer front daily, enough for that day's experiment. Buffer: 9.21 M NagOO, (21.2 g/L.)

4 MU Standard Stock: 4.96 mg MU in 25 ml df-(0 (made front daily) MU Substants Stock: 7 mg MU in 1.0 ml 95% ethanol (made front daily). iverage mM MU for each snaple (Mean Value Cohams) minutes reaction recorded – mM MU lm = 60 min lm = mM MU lm. Note that if there is 10 mS = mM MU lm = 80 mS = Corn sood extracts should already be prepared and analyzed for total protein according to standard procedures. in a staction plate, equilibrate up-to-to-paged total protein in a total
welcome of 100 gd lysis buffer. Generally samples can be analyzed with
I ag total protein. Samples should be analyzed in ciplicate. 10 pl of 1 tild MLI standard stock is diluted with 90 pl typis buffer. 10 pl of this 1:10 dilution is further diluted with 90 pl typis buffer to give a 1:100 dilution. EXHIBIT 0 ctd MU standard
1000 sM MU standard
10,000 sM MU standard X RESULTS: DATA FOUND BELOW. (10-MIN READENGS) Sample JAMPHE# 90 TSP Sample # 70 TSP 70TSP GG 01040-1 **-** 0 CSG 01110-1 D.6 0.06 65001060-1 A.4 0.04 G.H 0.04 -2 8 0.06 -3 -3 A 0 -3 0 ~4 6.5 0.05 8.4° 0.04 8.0 0.04 -5 0.4 0.04 48 0.5 -5 63D 02130 - 1 tot 0.1 42 04 GSC 01130-1 GSC 01070 -1 8.4 0.8 6.7 O.07 -2 27 0.3 out oui -3 0.9 0.1 -3 8.6 0.9 3.4 0.3 -3 -4 4  $\circ$ -4 5.0 0.5 Six 0.5 -4 0.001 -608 0.001 -5 0.8 C.1 -5 -5 0.07 696 01020-1 0 0 6SC 01040 -1 0.1 ODI G8C 01110-1 A 0 -2 <del>0</del>-0 5H 0.5 - Z -2 9.2 0.9 -3 O.12-0.01 - 3 0.3. 1.03 -3 D- 0 -4 <del>0</del> -4 O 0.03 -4 4 -5 0.02 0.04-0.004 76-07 -5 GSC 01030-1 · - 0 Bbok # 67 :2 4.0-0.4

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